Original Research Article

Rapid method of cytology diagnosis by supravital staining in FNAC of various tissue and organs

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Abstract

Background: Supravital staining is the staining of living tissue removed from the body, but before cessation of the chemical life of the cells. **Objective**: The present study has been undertaken with the aim of to assess adequacy of material during FNAC, study the cytomorphological features and rapid diagnosis by wet smears. Also, to evaluate the diagnostic capability of supravital stain applied over the tissue obtained by FNAC of various organs and aspirated body fluids. **Material and Method**: In present study 100 fine needle aspiration of various tissue and organ were examined for cytological evaluation. FNAC were done in the out patients and in patients of several departments of Gajara Raja Medical College. Out of 100 cases 33 from lymphnode, 19 from soft tissue swelling, 13- from breast lump, 13 from thyroid, 04 from parotid swelling, 14 from the body fluid and 04 miscellaneous. **Result**: Out of 100 cases 93 cases (93%) were diagnosed correctly and discrepancy of 7 cases found in cytological diagnosis by toluidine blue and H & E stain. Out of 48 non-neoplastic cases 45 diagnosed correctly with the overall accuracy of 93.75% and out of 52 neoplastic cases 48 were diagnosed correctly with the accuracy of 92.30%. **Discussion:** The advantage of this technique is that cells are seen in living natural condition without any artefact caused by fixation, air dry or cutting.

Key words: FNAC, Supra vital stains,Toluidine Blue, Haematoxylin and Eosin.

Introduction

Fine needle aspiration is an accurate and cost-effective tool used in Morden pathology also it has become one of the important methods for obtaining rapid diagnosis of lesions of many organs. A rapid intraoperative or preoperative diagnosis helps the surgeon to monitor and modify the approach of surgery [1].

Supravital staining is amethod in that a drop of sediment mix with a drop of staining solution in a fresh and unfixed sampletoguide cytotechnologist and demonstrate structures of living cells in wet preparation [2]. FNA material is stained with toluidine blue in wet preparation and conventional stain in fixed smear for microscopic examination to reach a proper diagnosis [3]. Wet mount study of FNAC establish three-dimensional view of cells, minimizing the smearing and drying artefact, loss of cells sample during fixation and improves diagnostic accuracy [4]. Supravital staining with toluidine blue in a fresh, unfixed sample can provide information to see the adequacy of material during FNAC. If material is found inadequate, the procedure can be repeat immediate to avoid unnecessary delay of report.

This can be routinely use as to improve the cellularity and reduce the time taken for re-sampling [5]. The technique is simple, rapid, easy and cost effective [6,7].

Material and Methods

This is prospective study conducted in the department of Pathology Gajra Raja Medical College from Aug 2003 to June 2004.

A total number of 100 FNA and body fluid samples has been taken which were referred to the outpatient department of cytology.

Out of 100 cases 33 from lymphnode, 19 from soft tissue swelling, 13 from breast lump, 13 from thyroid, 04 from parotid swelling, 14 from the body fluid and 04 are other sites.

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Inclusion criteria

1. Details of patient's identification, clinical history, provisional diagnosis, local and systemic examination, relevant radiological findings and previous report of FNAC or histopathology if done has been obtained.

2. A clear explanation of the procedure will ensure the patients consent and co-operation.

3. After aspiration careful examination has been done particularly to see texture of the tissue, presence of haemorrhage and necrosis.

4. Body fluid aspiratesmears were prepared after physical examination and centrifugation.

5. Wet smear is made from the part of aspirate or sediment mixed with supravital stain (0.5% Toluidine blue) to see the adequacy of material. If obtained material is adequate than alcohol fixed smears prepared and stained with Haematoxylin and Eosin stain.

Exclusion criteria- Ifdelay in staining after collection of samples and yield very little material.

Staining Method

Wet film – Supravital Stain
Alcohol fixed Stain – Haematoxylin and eosin (H & E) stain.

Supravital stain – 0.5% Toluidine Blue

Staining Technique

1. Put a drop of aspirate in the centre of slide.

2. Place a drop of toluidine blue mixed it with wooden applicator stick and cover it with coverslip.

3. Let sample set for a minute and evaluate under the microscope.

Toluidine blue stains cells blue-purple, provides good nuclear details with easily visualized three-dimensional formation in wet preparation and prominent vacuoles. The granules of basophil and mast cells stains bright red and purple.

Statistic Method- The p value obtained by chi square test

Result

In the present study a total number of 100 cases were subjected to smear diagnosis by FNAC of various tissues and organs. Distribution of cases according to site and lesions is given in the table no. 1

Table No.-1: Distribution of cases according to site.

S. No	Site of FNAC	No. of Cases
1.	Lymphnode	33
2.	Soft tissue	19
3.	Breast	13
4.	Thyroid	13
5.	Parotid	04
6.	Body fluid	14
7.	Other	04

The table No. 2 depicts the comparative study of non-neoplastic lesions of various tissue and organs stained by toluidine blue and H & E.

Out of 48 non-neoplastic cases 45 were diagnosed correctly on cytological study by supravital stain (Toluidine Blue) with overall accuracy of 93.75%. Out of 13 cases of chronic nonspecific lymphadenitis 11 cases were diagnosed correctly given an accuracy of 84.61%.

Out of 7 tubercular lesions of lymphnodes 6 cases were diagnosed correctly given an accuracy of 85.71%. Rest of lesions were diagnosed correctly on cytological diagnosis by wet smear stained with toluidine blue.

Table No.-2: Comparison between the Non-Neoplastic Lesion.

FNAC Site	Non-neoplastic lesions	Supravital Stain (Toluidine Blue)	H & E		Accuracy
			No. of Concordance Cases	No. of discordance cases	
Lymphnode	Chronic nonspecific or reactive lymphadenitis	13	11	2	84.61%
	Tuberculosis	07	06	1	85.71%
	Abscess	06	06	-	100%
Breast lump	Abscess	03	03		100%
Soft tissue swelling	Inflammatory lesions	02	02	-	100%
Body fluids	Ascitic fluid-inflammation	06	06	-	100%
	Fluid from liver cyst – Hydatid scolex	01	01	-	100%
	Bronchial aspiration-inflammation	05	05	-	100%
Parotid	Inflammatory Condition	01	01	-	100%
Others	Epidermoid Cyst	03	03	-	100%
	Cholestatic Jaundice	01	01	-	100%
	Total No. of Cases	**48**	**45**	**3**	**93.75%**

Table No. 3 depicts the comparative study of neoplastic lesions of various tissues and organs in wet smears stained by toluidine blue and H & E stain.

Table No.-3: Comparison between Neoplastic lesions.

FNAC Site	Neoplastic lesions	Toluidine Blue	H&E		Accuracy
			No. of Concordance Cases	No. of discordance cases	
Lymphnodes	Lymphoma	03	02	01	66.66%
	Secondaries	04	04	-	100%
Thyroid	Benign	13	13	-	100%
Soft tissue	Benign	04	04	-	100%
	Malignant	13	12	1	92.30%
Breast lump	Benign	07	06	1	85.71%
	Malignant	03	02	1	66.66%
Parotid	Pleomorphic adenoma	02	02	-	100%
	Mucoepidermoid Ca.	01	01	-	100%
Body fluid	Malignant	02	02	-	100%
Total No. of Cases		**52**	**48**	**04**	**92.30%**

Out of 52 neoplastic cases 48 were diagnosed correctly with an overall accuracy of 92.30%. Out of 13 cases of malignant soft tissue tumour of 12 cases were diagnosed correctly with an accuracy of 92.30%. Out of 7 cases of benign breast lump 6 cases were diagnosed correctly with an accuracy of 85.71%. Out of 3 malignant lesion of breast 2 cases were diagnosed correctly giving an accuracy of 66.66%. Out of 3 cases of lymphoma 2 cases were diagnose correctly with the accuracy of 66.66%. Rest of lesions were diagnosed correctly on cytological diagnosis in wet smear stained by toluidine blue.

Overall accuracy in neoplastic and non-neoplastic lesions is depicted in table no.4.

Table No.-4: Showing diagnostic accuracy of non-neoplastic and neoplastic lesions.

		Total No. of Cases Toluidine Blue	Total No. of cases in H &E		Accuracy
			Concordance	Discordance with T.B.	
Non-Neoplastic Lesions		48	45	03	93.75%
Neoplastic	Benign lesions	26	25	01	96.15%
	Malignant lesions	26	23	03	88.46
Total No. of Cases		**100**	**93**	**07**	**93%**

The chi-square statistic is 1.2614. The p-value is 0.532226. the result is not significant at $p<0.05$.

Out of 100 cases 93 cases (93%) diagnosed correctly, there was discrepancy of 7 cases in cytological diagnosis by toluidine blue and H & E. Histological confirmation available for discrepancy cases.

Discussion

The present study has been undertaken to study the cytomorphology of the frequently encountered lesions of various tissue and organs of body, examined by supravital staining in wet smear, to point out the problem and limitation in interpretation as well asto evaluate the usefulness of offering a rapid diagnosis to operating surgeon.

Cytological diagnosis has become one of the important tools for obtaining rapid diagnosis of lesions of many organs. In many instances it has been utilized for intraoperative diagnosis. A rapid intraoperative or preoperative diagnosis helps the surgeon to monitor and modify the approach in surgery [1]. Frozen section study which is popular amongst the surgeon for obtaining rapid intraoperative diagnosis has been not used for those organs from where the biopsy material is too soft, fragmented and not satisfactory for freezing. Frozen section technique is costly and requires technical expertise [2].

Wilkerson and Bonnin compared the diagnostic accuracy and the quality of specimens obtained in a series of cases studied by both intraoperative cytology and frozen section. They concluded the accuracy of diagnosis by both the techniques was not significantly different but the quality of cytologic preparation was significantly superior to that of frozen section [8].

As far as staining technique is concerned wet film preparation stained with one of the supravital stain has been used successfully by (Taft & Landlum 1930) with excellent result. Dudgeon & Patric recommended wet film technique for inflammatory and neoplastic lesion [9]. Drothy S Russel used wet film for diagnosis of tumour and inflammatory lesion in 60 cases and they observed that the wet film examination gives better morphological details [10]. Dinda etal., determined the role of supravital staining of urine sediment and bright field microscopy in diagnosis of acute renal failure in clinical medicine. The stain consists of 1% crystal violet and 0.5% safranin in normal saline and examined 32 cases of ARF in their initial presentation of oliguric phase [11].

In present study we have used 0.5% of toluidine blue with 20 ml of 95% ethyl alcohol and 80 ml of distilled water, and it gives very good result. Toluidine blue provides good, nuclear details with easily visualized three-dimensional formation and prominent cytoplasmic vacuoles. In wet mount preparation we can easily watch the movable parasite which are stained with toluidine blue. Scolex of hydatid mole and crystals are also seen in wet preparation. Lymphocytes stained dark bluish in colour with course chromatin and rim of bluish cytoplasm. NHL smears examined contains monotonous population of lymphoid cells shows slightly larger nucleus. In fibroadenoma smears cell showregularly arranged benign epithelial cells, round to oval nuclei having finely granular chromatin. Smears from pleomorphic adenoma show the mesenchymal fragments appears purple in colour, fibrillary, mucoid substance with well-defined rounded epithelial cells in sheets and few spindled myoepithelial cells. FNA from thyroid nodule shows light to dark purple colloid with follicular cells dispersed in small clusters. In some cases, macrophages and cholesterol crystals noted.Squamous cell carcinoma shows clusters of cells with dark blue cytoplasm having irregular angular hyperchromatic nuclei. Background shows necrosis.

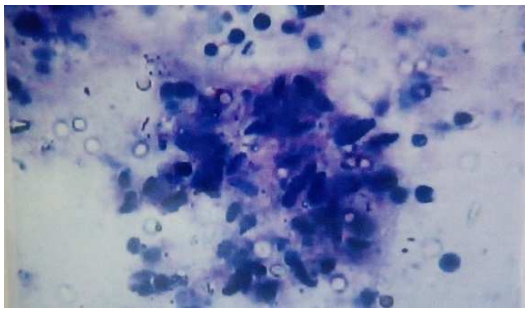

Fig. A: Epitheloidcells (TB Lymphnode)

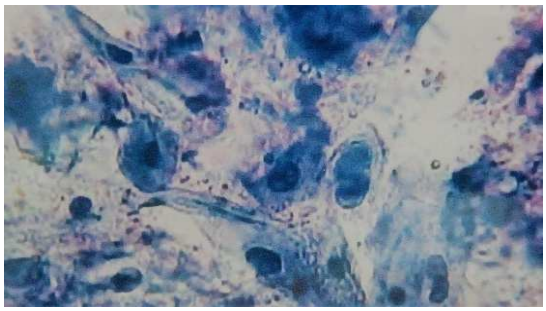

Fig B: Atypical Squamous epithelial cell

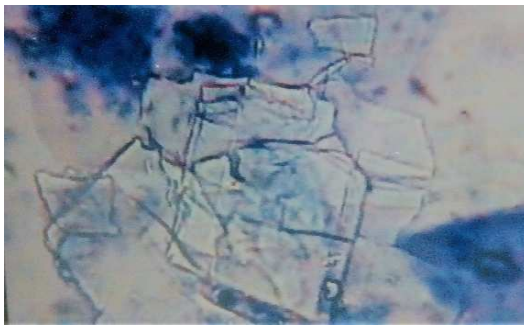

Fig.C: Cholesterol crystal

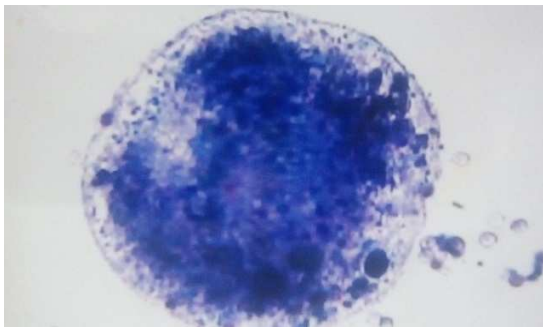

Fig D: Scolex of Hydatid cyst

If inadequate material is aspirated than further aspiration can be performed without any delay and this is reducing the time limit and improve the cellularity. Total 100 cases were subjected to wet preparation stained with toluidine blue and compared with fixed smear stained with H&E. Out of these 93 cases were diagnosed with the accuracy of 93%. Out of 48 non-neoplastic cases 45 (93.75) were diagnosed correctly. Out of 52 neoplastic lesions both in benign and malignant 48 (92.30%) cases were assessed correctly by wet preparation. There was discrepancy of 7 cases aspirated from lymphnode, soft tissue and breast. Toluidine blue is an acidophilic dye of thiazine group which stains acidic tissue components. As dysplastic and pleomorphic cells nucleic acid contain is more than normal cells, also malignant cells may contain wider intracellular canals would enhance penetration of the dye. Few investigators applied toluidine blue in vivo as a clinical indicator of premalignant and malignant lesions of oral cavity [12,13].

Mc Cormark CJ et al., have used 1% concentration of toluidine blue for identification of neoplastic cells in CSF by wet film method [14]. T Muller has done methylene blue supravital staining to evaluate its applicability in the mammalian brain and penial gland [15]. Joy MP etal., performed rapid diagnosis with toluidine stain in 295 ultrasound guided aspirates and found 98.54% sensitivity and 97.99% specificity in malignant/ suspicious for malignant cases. Sensitivity and specificity for an inflammatory lesion was 100% [16]. Cytologic preparation provide a useful diagnostic tool and plays a great role in the intraoperative diagnosis of CNS tumours to guide neurosurgeons [17,18]. The method is accurate, simple, rapid and cheap [16,17,18]. The advantage of this technique is that cells are seen in living natural condition without any artefact caused by fixation, air dry or cutting [19].

Conclusion

In present study cytological examination in wet cell preparation of various tissue is simple, cost effective, accurate and rapid technique which providesadequacy of aspirated material, so that a repeat aspiration can be done immediately to avoid inconvenience of patient.

Main disadvantage is that the smear cannot preserve for permanent record. This can be overcome by making fixed smear of same material and stained by conventional staining for keeping a permanent record. The present study is a pilot study and its utility in routine procedure needs to be further assessed. It requires study of large series from different organs, which will establish this procedure in routine cytological technique.

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Permission from IRB: Yes

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